IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Timothy D. Osslund		
Serial No.:	Not Yet Assigned	Group Art Unit No.:	1646
Filed:	Concurrently herewith	Examiner:	N. Basi
For:	G-CSF ANALOG COMPOSITIONS AND METHODS		
Docket No.:	A-231G		

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the referenced application as follows:

In the Specification:

On Page 1 please delete the first paragraph and replace it with the following new paragraph:

Cross Reference to Related Applications

The present application is a divisional of U.S. Patent Application No. 09/754,532, filed January 3, 2001, which is a divisional of U.S. Patent Application No. 09/304,186 , filed May 3, 1999, which is a continuation of U.S. Patent Application No. 09/027,508, filed February 20, 1998, which is a continuation of U.S. Patent Application No. 08/956,812, filed October 23, 1997, which is a divisional of U.S. Patent Application No. 08/448,716, filed May 24, 1995, now Patent No. 5,790,421, which is a divisional of U.S. Patent Application No. 08/010,099, filed January 28, 1993, now Patent No. 5,581,476, which are hereby incorporated by reference.

EXPRESS MAIL CERTIFICATE

"Express Mail" mail labeling number. EL198797496US Date of Deposit: December 20, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

S.L. St. Andrew
Printed Name

S.L. St. Andrew
Signature

In the Claims:

Please delete claim 1 and add new claims 60 to 61 as follows:

60. (New) A G-CSF analog, optionally with an N-terminal methionine, where in the amino acid sequence differs from the Figure 1 in that

(a) the lysines at positions 17, 35 and 41 are substituted with arginine; and

(b) the AB loop and CD loop are altered to include one or more lysines containing at least one polyethylene glycol molecule.

61. (New) A G-CSF analog, optionally with an N-terminal methionine, where in the amino acid sequence differs from Figure 1 in that

(a) the lysines at position 17, 35 and 41 are substituted with arginine; and

(b) the AB loop, BC loop and CD loop are altered to include one or more lysines containing at last one polyethylene glycol molecule.

Remarks

Applicant has amended paragraph one of the specification and attached is a separate page showing the marked up version entitled "Version with markings to show changes made". Applicant has deleted claim 1 and added new claims 60 to 61. Support for the new claims can be found in the specification as follows (based on the specification of the issued Patent No. 5,581,476):

Column 1, lines 8 to 10:	This invention related to granulocyte colony stimulating factor ("G-SF") analogs, compositions containing such analogs, and related compositions.
Column 4, lines 20 to 29:	Based on the correlation of structure to biological activity, one aspect of the present invention relates to G-CSF analogs. These analogs are molecules which have more, fewer, different or modified amino acid residues from the G-CSF amino acid sequence. The modificatons may be by addition, substitution, or deletion of one or more amino acid residues. The modification may include the addition or substitution of analogs of the amino acids themselves, such as peptidomimetics or amino acids with altered moieties susch as altered side groups.
Column 4, lines 37 to 40:	For example,the analogs may be designed to have a higher or lower biological activity, have a longer shelf-life or a decrease in stability, be easier to formulate, or more difficult to combine with other ingredients.
Column 4, lines 48 to 57:	In another aspect, the present invention relates to related compositions containing a G-CSF analog as an active ingredient. The term, "related composition", as used herein, is meant to denote a composition which may be obtained once the identify of the G-CSF analog is ascertained (such as a G-CSF analog labeled with a detectable label, related receptor or pharmaceutical composition). Also considered a related composition are chemically modified versions of the G-CSF analog, such as those having attached at least one polyethylene glycol molecule.
Column 5, lines 32 To 51:	Another example of a related composition is a G-CSF analog with a chemical moiety attached. Generally, chemical modification may alter biological activity or antigenicity of a protein, or

may alter other characteristics, and these factors will be taken into account by a skilled practitioner. As noted above, one example of such chemical moiety is polyethylene glycol. Modification may include the addition of one or more hydrophilic or hydrophobic polymer molecules, fatty acid molecules, or polysaccharide molecules. Examples of chemical modifiers include polyethylene glycol, alklpolyethylene glycols, DI-poly(amino acids), polyvinylpyrrolidone, polyvinyl alcohol, pyran copolymer, acetic acid/acylation, proprionic acid, palmitic acid, stearic acid, dextran, carboxymethyl cellulose, pullulan, or agarose. See, Francis, *Focus on Growth Factors* 3: 4-10 (May 1992)(published by Mediscript, Mountview Court, Friern Barnet Lane, London N20 OLD, UK). Also, chemical modification may include an additional protein or portion thereof, use of a cytotoxic agent, or an antibody. The chemical modification may also include lecithin.

Column 8, lines 7 to 17:

Therefore, another class of G-CSF analogs provided herein is that having an altered external loop but possessing the same overall structure as (non-altered) natural or recombinant G-CSF. More particularly, another class of G-CSF analogs provided herein are those having an altered external loop, said loop being selected from the loop present between helices A and B; between helices B and C; between helices C and D; between helices D and A, as those loops and helices are identified herein. More particularly, said loops, preferably the AB loop and/or the CD loop are altered to increase the half life of the molecule by stabilizing said loops.

Column 8, lines 34 to 49:

Additionally, such external loops may be the site(s) for chemical modification because in (non-altered) natural or recombinant G-CSF such loops are relatively flexible and tend not to interfere with receptor binding. Thus, there would be additional room for a chemical moiety to be directly attached (or indirectly attached via another chemical moiety which serves as a chemical connecting means). The chemical moiety may be selected from a variety of moieties available for modification of one or more function of a G-CSF molecule. For example, an external loop may provide sites for the addition of one or more polymer which serves to increase

	serum half-life, such as a polyethylene glycol molecule . Such polyethylene glycol molecule(s) may be added wherein said loop is altered to include additional lysines which have reactive side groups to which polyethylene glycol moieties are capable of attaching.
Column 8, lines 58 to 62:	Therefore, another class of the present G-CSF analogs includes those with at least one alteration in an external loop wherein said alteration provides for the addition of a chemical moiety such as at least one polyethylene glycol molecule.
Column 11, lines 20 to 32:	General objectives in chemical modification may include improved half-life (such as reduced renal, immunological or cellular clearance), altered bioactivity (such as altered enzymatic properties, dissociated bioactivities or activity in organic solvents), reduced toxicity (such as concealing toxic epitopes, compartmentalization, and selective biodistribution), altered immuno-reactivity (reduced immunogenicity, re-duced antigenicity or adjuvant action), or altered physical properties (such as increased solubility, improved thermal stability, improved mechanical stability, or conformational stabilization). See Francis, *Focus on Growth Factors* 3: 4-10 (May 1992)(published by Mediscript, Mountview Court, Friern Barnet Lane, London N20 OLD, UK).
Column 22, lines 24 to 29:	Set forth below are the oligonucleotides used for each G-CSF analog prepared via the M13 mutagenesis method. The nomenclature indicates the residue and the position of the original amino acid (e.g., Lysine at position 17), and the residue and position of the substituted amino acid (e.g., arginine 17).
Columns 21-22, 23-24:	Table 2
Columns 27-28:	Table 4
Columns 29-30:	Table 5
Column 32, lines 24 to 29:	The above biological activity data, from the presently prepared G-CSF analogs, demonstrate that modification of the external loops interfere least with G-CSF overall structure. Preferred loops for

analog prepration are the AB loop and the CD loop. The loops are relatively flexible structures as compared to the helices.

Column 32-33, lines 63-10: Thus, by alteration of the external loops, preferably the AB loop (amino acids 58-72 of r-hu-met G-CSF) or the CD loop (amino acids 119 to 145 of r-hu-met-G-CSF), and less preferably the amino terminus (amino acids 1-10), one may therefore modify the biological function without elimination of G-CSF G-CSF receptor binding. For example, one may : (1) increase half-life (or prepare an oral dosage form, for example) of the G-CSF molecule by, for example, decreasing the ability of proteases to act on the G-CSF molecule or adding chemical modifications to the G-CSF molecule, such as one or more polyethylene glycol molecules or enteric coatings for oral formulation which would act to change some characteristic of the G-CSF molecule as described above, such as increasing serum or other half-life or decreasing antigenicity; ...

If Applicant has overlooked any fee or petition associated with this paper, the Commissioner is authorized to charge any such fee to deposit account No. 01-0519, and please consider such appropriate petition. Any questions associated with this application may be directed to Applicant's attorney, (805) 447-6885.

Please send all future correspondence to

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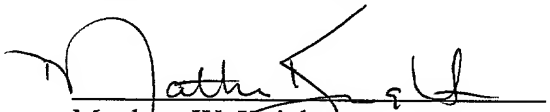
Respectfully submitted,



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Version with markings to show changes made

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